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Reduced Protective Efficacy of a Blood-Stage Malaria Vaccine by Concurrent Nematode Infection

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Helminth infections, which are prevalent in areas where malaria is endemic, have been shown to modulate immune responses to unrelated pathogens and have been implicated in poor efficacy of malaria vaccines in humans. We established a murine coinfection model involving blood-stage *Plasmodium chabaudi* AS malaria and a gastrointestinal nematode, *Heligmosomoides polygyrus*, to investigate the impact of nematode infection on the protective efficacy of a malaria vaccine. C57BL/6 mice immunized with crude blood-stage *P. chabaudi* AS antigen in TiterMax adjuvant developed strong protection against malaria challenge. The same immunization protocol failed to induce strong protection in *H. polygyrus*-infected mice. Immunized nematode-infected mice produced significantly lower levels of malaria-specific antibody than nematode-free mice produced. In response to nematode and malarial antigens, spleen cells from immunized nematode-infected mice produced significantly lower levels of gamma interferon but more interleukin-4 (IL-4), IL-13, and IL-10 in vitro than spleen cells from immunized nematode-free mice produced. Furthermore, *H. polygyrus* infection also induced a strong transforming growth factor β1 response in vivo and in vitro. Deworming treatment of *H. polygyrus*-infected mice before antimalarial immunization, but not deworming treatment after antimalarial immunization, restored the protective immunity to malaria challenge. These results demonstrate that concurrent nematode infection strongly modulates immune responses induced by an experimental malaria vaccine and consequently suppresses the protective efficacy of the vaccine against malaria challenge.

Helminth infections, which are prevalent in many regions of the world, cause various morbidities in humans including reduced fitness, malnutrition, retarded growth, and anemia (7, 8, 25, 41). In addition, studies with humans and animal models have demonstrated that helminth parasites modulate the immune responses to unrelated pathogens or antigens, which often results in impaired protective immunity to the pathogens (1, 4, 20, 23). For example, patients infected with *Ascaris lumbricoides* exhibit lower interleukin-2 (IL-2) and gamma interferon (IFN-γ) cytokine responses to cholera vaccine than worm-free individuals exhibit (9). Blood mononuclear cells from patients with filarial infections are more susceptible to human immunodeficiency virus infection and replication than cells from control subjects are (16). In addition, immunization with *Mycobacterium bovis* BCG vaccine induces a lower level of protection against virulent *Mycobacterium tuberculosis* challenge in *Schistosoma mansoni*-infected mice than in *S. mansoni*-free mice (12).

Helminth infections coexist with malaria in many parts of the world and have been shown to modulate the development of host protective immunity to natural malaria infection. Independent studies in Thailand and Senegal demonstrated that individuals harboring helminth parasites have a higher risk of malaria attack than individuals who are worm-free (27, 34). A recent study showed that mice coinfected with the filarial parasite *Litomosoides sigmodontis* and blood-stage *Plasmodium chabaudi* had more severe malaria disease than mice infected with malaria alone had (17). These observations indicate that a preexisting helminth infection strongly modulates the development of immune protection and immunopathology in the host following a primary malarial infection.

Vaccination is considered to be the most efficient approach to confer protection against malaria. However, vaccine trials conducted in areas where malaria is endemic often fail to induce effective protection (2, 15). Many factors are believed to be responsible for the poor efficacy of malaria vaccines; these factors include genetic polymorphism of the human population, malnutrition, an immature immune system in young children, and the complex life cycle and antigenic variation of the malaria parasite (5, 14, 33, 35). Since helminth parasites exacerbate primary malarial infection (27, 34), it has been hypothesized that coexistence of a helminth infection in areas where malaria is endemic may be an important confounding factor that impedes the development of vaccine-induced protective immunity against malaria (26).

We established a murine model of coinfection with a nematode parasite, *Heligmosomoides polygyrus*, and blood-stage *P. chabaudi* AS to investigate the immunomodulatory effect of a concurrent helminth infection on immune responses to blood-stage malaria parasites. We demonstrated previously that concurrent *H. polygyrus* infection makes otherwise resistant C57BL/6 mice highly susceptible to primary *P. chabaudi* AS infection and alters a number of key immunoregulatory cytokine and antibody responses to malaria (39). In the present study, we used this gastrointestinal nematode-malaria coinfection model to test the hypothesis that concurrent helminth

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parasitic infection impairs vaccine-induced protective immunity against malaria challenge.

MATERIALS AND METHODS

Mice, parasites, and experimental infection. Age-matched female mice that were 8 to 10 weeks old were used in all experiments. C57BL/6 and BALB/c mice were purchased from Charles River Laboratories (St. Constant, Quebec, Canada). Mice were maintained in the animal facility of the Research Institute of the McGill University Health Centre (Montreal, Quebec, Canada) under specific-pathogen-free conditions. The blood-stage *P. chabaudi* AS malaria parasite was maintained in mice by weekly passage as previously described (31). Blood-stage malaria challenge was initiated by intraperitoneal (i.p.) injection of 10^6 *P. chabaudi* AS-parasitized red blood cells (pRBC). Malaria parasitemia was monitored by using blood smears stained with Diff-Quik (American Scientific Products, McGraw Park, IL). *H. polygyrus* was kindly provided by M. Scott (McGill University, Montreal, Canada) and was maintained in BALB/c mice as previously described (39). Mice were infected by oral inoculation with 200 *H. polygyrus* third-stage larvae.

Antimalarial immunization. Whole blood-stage *P. chabaudi* AS antigen was prepared by using a modification of a freeze-thaw protocol described previously (38). For immunization, 10 μl of crude antigen (equivalent to 10^7 pRBC) was diluted with 40 μl of phosphate-buffered saline (PBS) and emulsified with 50 μl of TiterMax adjuvant (CytRx Corporation, Norcross, GA). One hundred microliters of the emulsified mixture was injected subcutaneously in the nape of each mouse. Three weeks later, immunized mice were boosted by i.p. injection of the same amount of antigen in 0.1 ml PBS without adjuvant. Two weeks after boosting, mice were either challenged with 10^6 pRBC or sacrificed for analysis of the cytokine responses in vivo and in vitro.

Anthelmintic drug treatment. To terminate *H. polygyrus* infection, mice were treated orally with pyrantel pamoate (100 mg/kg of body weight) (kindly provided by M. Gottschalk, Université de Montréal, St. Hyacinthe, Canada) (3).

Spleen cell cultures. Spleens from normal and infected mice were removed, and single-cell suspensions were prepared in RPMI 1640 medium (Gibco, Burlington, Ontario, Canada) supplemented with 10% heat-inactivated fetal calf serum (HyClone Laboratories, Logan, UT), 25 mM HEPES (Gibco), 0.12% gentamicin (Schering, Montreal, Canada), and 2 mM glutamine (Gibco) (complete medium). Spleen cells (5×10^6 cells/ml) were cultured in 48-well culture plates in complete medium alone, in the presence of pRBC (10^6 cells/ml) as a source of malaria antigen, in the presence of *H. polygyrus* adult worm antigen (20 μg/ml) prepared as previously described (39), or in the presence of both malaria and nematode antigens. The cultures were incubated for 48 h at 37°C in a humidified CO_2 incubator. Supernatants were collected and stored at −20°C until they were assayed to determine cytokine levels.

Cytokine ELISAs. Levels of IFN-γ, IL-4, and IL-10 in cell culture supernatants were determined by sandwich enzyme-linked immunosorbent assays (ELISAs) using paired capture and detection antibodies (BD Biosciences, San Diego, CA) as previously described (36, 37). The concentrations of IL-13 in culture supernatants were determined using an ELISA kit (R&D Systems, Minneapolis, MN). The levels of total transforming growth factor β1 (TGF-β1) in supernatants and bioactive TGF-β1 in plasma were determined by an ELISA using paired capture and detection rat monoclonal antibodies which recognize mouse, human, and pig TGF-β1 (BD Biosciences), as described previously (39).

***P. chabaudi* AS-specific antibody levels.** Serum levels of *P. chabaudi* AS-specific antibodies were determined by ELISAs as previously described (39). To detect total immunoglobulin (Ig) and IgG1, horseradish peroxidase-conjugated goat anti-mouse Ig and IgG1 antibodies (Southern Biotechnology Associates, Birmingham, AL) were used, respectively. To measure the IgG2a subclass in C57BL/6 mice, a horseradish peroxidase-conjugated goat polyclonal antibody specific for the $IgG2a^b$ allotype was used as the detecting antibody (Southern Biotechnology Associates) (24). The antibody levels in serum were expressed as endpoint titers, the reciprocal of the highest dilution that yielded the background optical density.

Statistical analysis. Repeated-measure analysis of variance (ANOVA) was performed to determine the significance of differences in overall parasitemia levels among experimental groups. Cytokine and antibody data are presented below as means ± standard errors. The nonparametric Mann-Whitney U test was used to test the significance of differences between experimental groups. A P value less than 0.05 was considered significant.

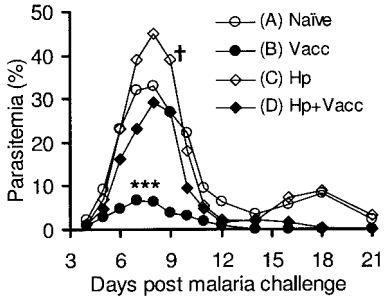

FIG. 1. Course of blood-stage *P. chabaudi* AS challenge infection in control and *H. polygyrus*-infected mice with and without antimalarial vaccination. Female C57BL/6 mice were infected with 200 *H. polygyrus* third-stage larvae (groups C and D). Two weeks after infection, a group of nematode-infected mice (group D) and a group of naïve mice (group B) were immunized with *P. chabaudi* AS antigen in TiterMax and boosted as described in Materials and Methods. Two weeks after boosting, all three groups of mice and a group of naïve control mice (group A) were infected i.p. with 10^6 *P. chabaudi* AS-parasitized red blood cells, and malaria parasitemia was monitored for 3 weeks. The data shown are mean parasitemia levels ($n = 5$); the standard errors (not shown) were less than 10% of the mean parasitemia for one of three replicate experiments which showed similar patterns of *P. chabaudi* AS challenge infection in all four groups of mice. Repeated-measure ANOVA was used to analyze the differences in overall parasitemia levels between groups. Mortality, indicated by a dagger, was calculated from data ($n = 13$) pooled from three experiments. Three asterisks indicate that the P value was <0.001 for a comparison with naïve mice in group A. Hp, *H. polygyrus*-infected mice; Vacc, vaccinated mice.

RESULTS

Reduced vaccine-induced protection against malaria in nematode-infected mice. We previously demonstrated that immunization of mice with a crude blood-stage *P. chabaudi* AS antigen in immunostimulatory adjuvants induces strong protection against challenge infection (38). In the present study, we used crude *P. chabaudi* AS antigen emulsified in TiterMax adjuvant as a malaria vaccine and the vaccination protocol described previously (38) to investigate the effect of nematode infection on vaccine-induced immune protection against malaria. The following four groups of C57BL/6 mice were used: (i) naïve control mice (group A), (ii) mice immunized with malaria vaccine (group B), (iii) mice infected with *H. polygyrus* (group C), and (iv) mice infected with *H. polygyrus* for 2 weeks and then immunized with malaria vaccine (group D). All four groups of mice were subsequently challenged with blood-stage malaria. The protocol of establishing an *H. polygyrus* infection for 2 weeks prior to vaccination was based on our observation that mice preinfected with *H. polygyrus* for 2 weeks have an impaired immune response to primary *P. chabaudi* AS infection (39). As shown in Fig. 1, immunization of nematode-free mice with malaria vaccine (group B) induced strong protective immunity against *P. chabaudi* AS infection, as demonstrated by low levels of parasitemia after malaria challenge. Compared with the parasitemia in nonimmunized control mice (group A), the overall levels of acute parasitemia from day 4 to day 12 after malaria challenge were significantly reduced ($P < 0.001$) and the peak parasitemia at day 8 was decreased by 80% in vaccinated mice (group B versus group A). As reported in our previous study (39), mice coinfected with *H. polygyrys* and *P.*

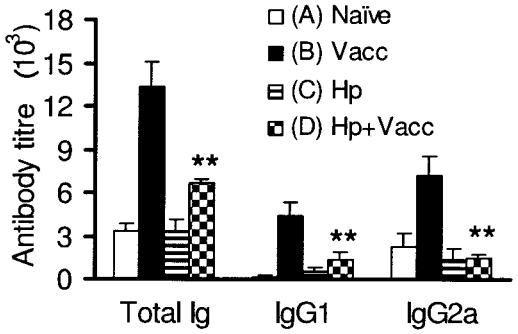

FIG. 2. *P. chabaudi* AS-specific total immunoglobulin, IgG1, and IgG2a antibody responses in control and *H. polygyrus*-infected mice with and without antimalarial vaccination. Groups of mice were infected with *H. polygyrus*, immunized with malaria antigen in TiterMax, and challenged with blood-stage *P. chabaudi* AS as described in the legend to Fig. 1. Ten days after malaria challenge, mice were sacrificed, and the levels of malaria-specific total Ig and antibody isotypes in sera were determined by ELISAs. The results are expressed as means and standard errors ($n = 4$) for one of two replicate experiments. Two asterisks indicate that the difference was significant ($P < 0.01$) for a comparison with vaccinated nematode-free mice. Hp, *H. polygyrus*-infected mice; Vacc, vaccinated mice.

chabaudi AS (group C) exhibited significantly higher levels of peak parasitemia at day 8 after primary *P. chabaudi* AS infection than mice infected with *P. chabaudi* AS alone exhibited ($P < 0.01$ for a comparison of groups C and A), and 30% of the coinfected mice died on day 9 after *P. chabaudi* AS infection (Fig. 1 and data not shown). The nematode-infected mice immunized with malaria vaccine (group D) all survived the *P. chabaudi* AS challenge and had a peak parasitemia level that was 30% lower than that of nematode-infected, nonimmunized mice (groups D and C), but the difference in overall parasitemia between these two groups was not statistically significant ($P > 0.05$). The parasitemia levels in nematode-infected immunized mice were as high as those in nematode-free nonimmunized mice ($P > 0.05$ for a comparison of groups D and A). These results demonstrate that the level of protective immunity induced by malaria vaccine is reduced in mice in the presence of a concurrent nematode infection.

Impaired antibody responses to antimalarial vaccination. We then measured the malaria-specific total Ig, IgG1, and IgG2a titers in the four groups of mice. Prior to malaria challenge, immunized nematode-free and nematode-infected mice produced low levels of malaria-specific antibody, and no significant differences were detected between the two groups (data not shown). Ten days after malaria challenge, immunized nematode-free mice produced increased levels of malaria-specific Ig, IgG1, and IgG2a (Fig. 2). The levels of malaria-specific Ig, IgG1, and IgG2a were significantly lower in immunized nematode-infected mice than in immunized nematode-free mice (groups D and B, respectively) (50%, 68%, and 80%, lower, respectively) (Fig. 2).

Altered cytokine responses in nematode-infected mice. To determine whether concurrent *H. polygyrus* infection modulates the pattern and/or levels of cytokines induced by antimalaria vaccination, cytokine production in vitro by spleen cells was analyzed (Fig. 3). Spleen cells from immunized nematode-free mice produced high levels of IFN-γ when they were cultured in the

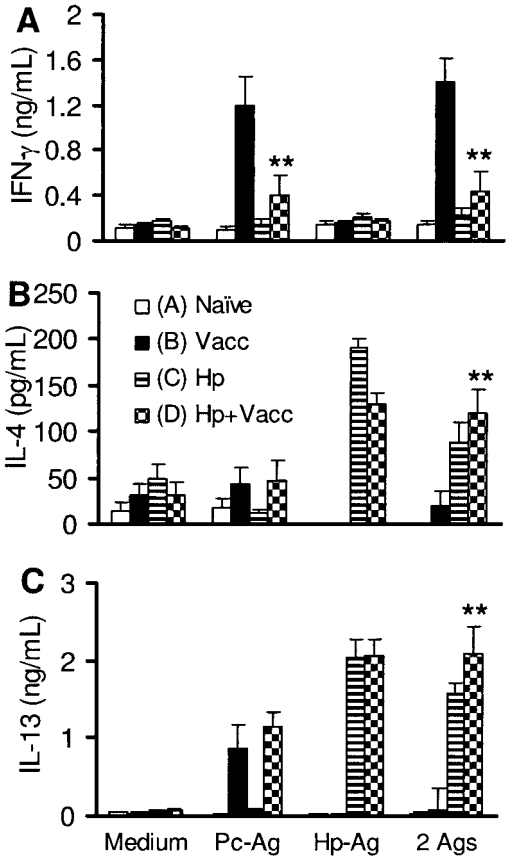

FIG. 3. In vitro cytokine production by spleen cells from normal control and *H. polygyrus*-infected mice with and without antimalarial vaccination. Mice were infected with *H. polygyrus* and immunized with malaria antigen in TiterMax as described in the legend to Fig. 1. Two weeks after boosting, mice were sacrificed, and spleen cells were cultured in medium or in the presence of pRBC as a source of *P. chabaudi* AS antigen (Pc-Ag), in the presence of *H. polygyrus* adult worm antigen (Hp-Ag), or in the presence of both antigens (2 Ags). The levels of IFN-γ (A), IL-4 (B), and IL-13 (C) were determined by ELISAs. The results are expressed as means and standard errors ($n = 4$) for one of two replicate experiments. Two asterisks indicate that the difference was significant ($P < 0.01$) for a comparison with vaccinated nematode-free mice with the same antigen stimulation. Hp, *H. polygyrus*-infected mice; Vacc, vaccinated mice.

presence of pRBC (Fig. 3A). Spleen cells from nematode-infected mice (groups C and D) produced little IFN-γ when they were cultured with nematode antigen. Importantly, spleen cells from immunized nematode-infected mice cultured in the presence of pRBC produced significantly lower levels of IFN-γ than cells from immunized nematode-free mice produced. Infection with *H. polygyrus* is known to induce a strong Th2-type cytokine response. Indeed, spleen cells from nematode-infected mice (groups C and D) produced high levels of IL-4 and IL-13 when they were stimulated with nematode antigen (Fig. 3B and C). Spleen cells from both nematode-free and nematode-infected mice immunized with malaria vaccine (groups B and D) also produced moderate levels of IL-13 when they were stimulated with pRBC (Fig. 3C).

We also analyzed the responses of the immunoregulatory cytokines IL-10 and TGF-β1 to antimalaria vaccination and *H.*

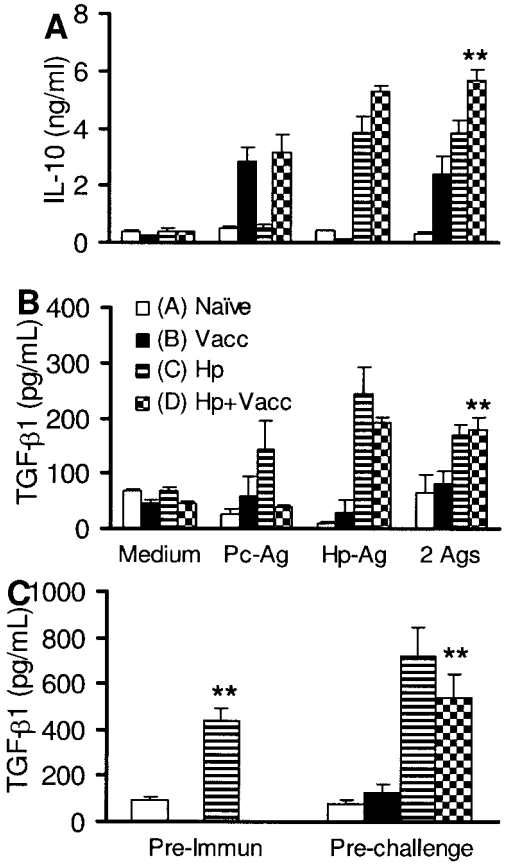

FIG. 4. In vitro IL-10 (A) and TGF-β1 (B) production by spleen cells and levels of bioactive TGF-β1 in plasma (C) from normal control and *H. polygyrus*-infected mice with and without antimalarial vaccination, as described in the legend to Fig. 1. (A and B) Spleen cells were collected from the four groups of mice and cultured in vitro as described in the legend to Fig. 3. Levels of IL-10 and total TGF-β1 were determined by ELISAs. The results are expressed as means and standard errors ($n = 4$) for one of two replicate experiments. Two asterisks indicate that the difference was significant ($P < 0.01$) for a comparison with vaccinated nematode-free mice with the same antigen stimulation. (C) Plasma samples were collected from normal mice and mice 2 weeks after *H. polygyrus* infection (Pre-immun) or from normal control and *H. polygyrus*-infected mice with and without antimalarial vaccination as described above (Pre-challenge), and levels of bioactive TGF-β1 in plasma were determined by ELISAs. The results are expressed as means and standard errors ($n = 4$) for one of two replicate experiments. Two asterisks indicate that the difference was significant ($P < 0.01$) for a comparison with either normal mice or vaccinated nematode-free mice. Vacc, vaccinated mice; Hp, *H. polygyrus*-infected mice; Pc-Ag, cells cultured in the presence of pRBC as a source of *P. chabaudi* AS antigen; Hp-Ag, cells cultured in the presence of *H. polygyrus* adult worm antigen; 2 Ags, cells cultured in the presence of both antigens.

polygyrus infection. As shown in Fig. 4A, spleen cells from immunized nematode-free and nematode-infected mice (groups B and D) produced increased levels of IL-10 when they were stimulated with pRBC. Spleen cells from nematode-infected mice (groups C and D) produced high levels of IL-10 when they were cultured with nematode antigen. In the presence of both pRBC and nematode antigen, cells from immunized nematode-infected mice produced a significantly higher level of IL-10 than cells from immunized nematode-free mice produced (groups D and B) (Fig. 4A), suggesting that *H. polygyrus* infection may enhance the IL-10 response to malaria vaccine. In addition, spleen cells from nematode-infected mice (groups C and D) produced high levels of total TGF-β1 when they were stimulated with nematode antigen, which was not observed in spleen cells from nematode-free mice (Fig. 4B). We also determined the TGF-β1 response to *H. polygyrus* infection in vivo by measuring the levels of bioactive TGF-β1 in plasma before both immunization and malaria challenge (Fig. 4C). Prior to immunization, mice preinfected with *H. polygyrus* produced significantly higher levels of bioactive TGF-β1 in plasma than naïve control mice produced, consistent with our previous findings (39). The groups of *H. polygyrus*-infected mice with and without antimalarial immunization (groups C and D) had high levels of bioactive TGF-β1 in plasma before malaria challenge, indicating that there was a high level of TGF-β1 in *H. polygyrus*-infected mice during the immunization period (Fig. 4C).

Deworming to improve the protective efficacy of malaria vaccine. The results described above demonstrated that concurrent *H. polygyrus* infection decreased the protective efficacy of malaria vaccine. To determine whether deworming improved the vaccine-induced antimalarial immunity in *H. polygyrus*-infected mice, four groups of C57BL/6 mice were infected with *H. polygyrus*. These mice were either not treated (group A), immunized with malaria vaccine (group B), immunized with malaria vaccine and treated with the anthelmintic drug during the last week of the immunization protocol (group C), or treated with the anthelmintic drug 1 week before immunization (group D), as shown in Fig. 5A. In a preliminary study, we determined the effect of the anthelmintic drug on the immune responses to malaria vaccine and found that drug treatment given 1 week before antimalarial immunization did not alter the levels of malaria parasitemia and antibody responses following *P. chabaudi* AS challenge (data not shown). Mice in groups A to D were challenged with 10^6 pRBC, and the course of parasitemia was monitored for 21 days. Consistent with results shown in Fig. 1, antimalarial immunization of nematode-infected mice (group B) slightly reduced the malaria parasitemia compared with that in the nematode-infected nonimmunized mice (group A), but the difference was not statistically significant (Fig. 5B). Mice treated with the anthelmintic drug at the end of malaria vaccination developed levels of malaria parasitemia similar to the levels that the mice that were not dewormed developed (groups C and B). In contrast, mice that were dewormed prior to antimalarial vaccination developed significantly lower levels of parasitemia than vaccinated mice that were not dewormed developed ($P < 0.001$ for a comparison of groups D and B), and the levels of parasitemia were similar to those observed in immunized nematode-free mice (group B) (Fig. 1). These results suggest that removal of nematode parasites before immunization, but not removal of nematode parasites after immunization, restores the ability of malaria vaccine to induce efficient protection against malaria challenge.

Effects of anthelmintic drug and adjuvant on the immune response to *H. polygyrus* infection. Since treatment of *H. polygyrus*-infected mice with the anthelmintic drug enhanced the protective immune response induced by the malaria vaccine, it was important to determine if cytokine responses were altered in *H. polygyrus*-infected mice following the deworming treatment. In addition, TiterMax was used as an adjuvant to

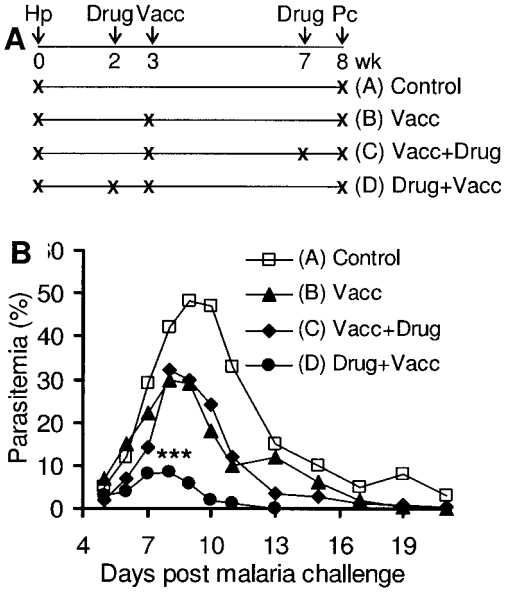

FIG. 5. Effect of anthelmintic treatment on the efficacy of a malaria vaccine. (A) Four groups of mice were infected with 200 *H. polygyrus* third-stage larvae (Hp). Two weeks after infection, mice in group D were treated with pyrantel pamoate (100 mg/kg of body weight) (Drug). Three weeks after nematode infection, mice in groups B, C, and D were immunized with malaria vaccine (Vacc) as described in Materials and Methods. In the last week of vaccination, mice in group C were treated with the anthelmintic drug. Mice in all four groups were challenged with 10^6 *P. chabaudi* AS-parasitized red blood cells (Pc). (B) Levels of parasitemia in the four groups of mice following *P. chabaudi* AS challenge infection. The data are mean parasitemia levels ($n = 4$); the standard errors (not shown) were less than 10% of the mean parasitemia levels. The results are the results of one of two replicate experiments which showed similar patterns of *P. chabaudi* AS challenge infection in the four groups of mice. Repeated-measure ANOVA was used to analyze the differences in overall parasitemia levels between groups. Three asterisks indicate that the *P* value was <0.001 for a comparison with group B.

enhance the immune response to the malaria antigen in the experiments described above. It was also important, therefore, to examine whether the adjuvant enhanced the immune response to the ongoing *H. polygyrus* infection, which in turn may have caused the impaired protective efficacy of the malaria vaccine in nematode-infected mice. To examine these possibilities, three groups of mice were infected with *H. polygyrus.* Two weeks later, one group of infected mice was left untreated, the second group was treated with the anthelmintic drug as described above, and the third group was inoculated with Titer Max at the same dose and by the same route that were used for antimalarial immunization. Two weeks later, these three groups of mice and a group of naïve mice were sacrificed. Intestines from *H. polygyrus*-infected mice were collected for determination of the worm burden, and spleen cells were cultured with *H. polygyrus* antigen for determination of cytokine production. As expected, anthelmintic drug treatment of *H. polygyrus*-infected mice removed all adult worms (data not shown). Importantly, spleen cells from the *H. polygyrus*-infected and drug-treated mice produced significantly lower levels of antigen-specific IL-4, IL-10, and TGF-β1 than spleen cells from the untreated *H. polygyrus*-infected mice produced (Table 1). Mice infected with *H. polygyrus* that were not treated with TiterMax and mice infected with *H. polygyrus* that were treated with TiterMax harbored similar numbers of adult worms (87 ± 8 adult worms for untreated mice; 84 ± 7 adult worms for TiterMax-treated mice; means ± standard deviations; $P > 0.05$). In addition, spleen cells from *H. polygyrus*-infected mice that were not treated with TiterMax and spleen cells from *H. polygyrus*-infected mice that were treated with TiterMax produced similar high levels of IL-4, IL-13, IL-10, and TGF-β1 when they were stimulated with nematode antigen (Table 1). These results indicate that TiterMax does not alter either the course of *H. polygyrus* infection or antigen-specific cytokine production.

DISCUSSION

Development of a vaccine to control malaria remains a major challenge. Although a number of protective antigens have been identified in human *Plasmodium* parasites, vaccine trials conducted in areas where malaria is endemic often yield poor results (2, 15). Among the factors that may decrease the protective efficacy of malaria vaccines, helminth parasite infections, which are very prevalent in areas where malaria is endemic, have been implicated as a confounding factor in the poor efficacy of malaria vaccines tested in humans (26). In this study, we used a murine nematode-malaria coinfection model to investigate the effect of concurrent nematode infection on the protective efficacy of an experimental malaria vaccine. We observed that nematode-free mice immunized with crude blood-stage *P. chabaudi* AS antigen in TiterMax developed strong protection against blood-stage malaria challenge infec-

TABLE 1. Effects of anthelmintic drug and TiterMax adjuvant treatment on the cytokine response to *H. polygyrus* infection[a]

Mouse group	Levels of cytokines (ng/ml)				
	IL-4	IL-13	IL-10	TGF-β1	IFN-γ
Control	0.01 ± 0.00	0.04 ± 0.01	0.22 ± 0.06	0.06 ± 0.02	0.28 ± 0.02
Hp	0.45 ± 0.05	2.95 ± 0.34	6.06 ± 0.30	0.55 ± 0.11	0.41 ± 0.08
Hp + Drug	0.13 ± 0.02[b]	2.32 ± 0.08	3.90 ± 0.26[b]	0.14 ± 0.12[b]	0.37 ± 0.09
Hp + TiterMax	0.41 ± 0.05	3.10 ± 0.20	5.16 ± 0.22	0.46 ± 0.10	0.39 ± 0.08

[a] Groups of female C57BL/6 mice ($n = 4$) were infected with 200 *H. polygyrus* third-stage larvae. Two weeks later, the nematode-infected mice were either not treated (Hp), treated with pyrantel pamoate (100 mg/kg of body weight) (Hp + Drug), or treated with TiterMax adjuvant (50 μl adjuvant emulsified in 50 μl PBS given subcutaneously) (Hp + TiterMax). Two weeks after drug or adjuvant treatment, the three groups of mice and a group of naïve control mice (Control) were sacrificed, spleen cells were cultured in the presence of *H. polygyrus* antigen (20 μg/ml), and the levels of cytokines in supernatants were determined by ELISAs. The data are means ± standard errors for one of two replicate experiments.

[b] $P < 0.05$ for a comparison with the value for mice-that were not treated.

tion, as demonstrated by the significant reduction in parasitemia in immunized mice. Although nematode-infected mice immunized with malaria vaccine also exhibited reduced parasitemia and improved survival, the magnitude of protection was much less than that observed for immunized nematode-free mice. The reduced vaccine-induced protective immunity in nematode-infected mice was not due to adjuvant-mediated alterations of immune responses to *H. polygyrus* infection because treatment of nematode-infected mice with TiterMax alone did not alter either the worm burden or the cytokine responses to *H. polygyrus*. These results demonstrate that concurrent nematode infection inhibits the development of vaccine-induced protective immunity against blood-stage malaria. Determining whether deworming can improve the efficacy of antimalarial vaccination has practical importance. Our results show that mice dewormed before antimalarial immunization, but not mice dewormed after antimalarial immunization, developed strong protective immunity in association with significant decreases in *H. polygyrus*-specific production of IL-4, IL-10, and TGF-β1. These results suggest that the reduced immune protection observed in immunized *H. polygyrus*-infected mice is mainly due to the immunosuppression caused by the nematode parasite during the vaccination period.

Antibody plays an important role in acquired protective immunity against blood-stage malaria either through inhibition of merozoite invasion of red blood cells or antibody-mediated cell cytotoxicity against infected red blood cells (6, 28). We have previously demonstrated that IgG2a antibody, a hallmark of the Th1-associated immune response, is the major isotype mediating immune protection against blood-stage *P. chabaudi* AS infection (37), a finding confirmed by other workers (13). We also observed that immunization of IFN-γ gene knockout mice failed to induce any protection against malaria infection (38), suggesting that IFN-γ plays a critical role in vaccine-induced immunity. In the present study, we observed that the immunized nematode-infected mice exhibited an impaired IFN-γ response to the malaria vaccine and produced significantly less malaria-specific antibodies than immunized nematode-free mice produced. The lower levels of antibody production, particularly IgG2a production, in nematode-infected mice may be responsible for their impaired ability to control a malaria challenge infection.

As shown by most helminth infections in humans and laboratory animals, infection with *H. polygyrus* induces a strong Th2-polarized immune response characterized by increased IL-4 and IL-13 production (Fig. 3), increased levels of serum IgE, and eosinophilia (18). It is generally believed that the Th2 cytokines induced by helminth infection suppress Th1-mediated effector mechanisms, leading to impaired immunity to intracellular pathogens (10, 22). Our results showing that the immunized nematode-infected mice exhibited strong IL-4 and IL-13 responses but impaired IFN-γ and IgG2a responses seem to support this scenario. However, despite the Th2-biased environment, these mice also exhibited a reduced Th2-associated IgG1 response to malaria antigen, suggesting that there may be a generalized immunosuppressive mechanism rather than Th1/Th2 antagonism in nematode-infected mice.

The immunosuppressive cytokines IL-10 and TGF-β play important roles in the maintenance of immunohomeostasis (11, 19). Primary blood-stage malaria infection in mice induces production of IL-10 and TGF-β1 (39), which have been shown to prevent malaria-associated immunopathology by down-regulating proinflammatory cytokine responses (21, 29). Due to their immunosuppressive effects, high levels of IL-10 and TGF-β1 have been shown to inhibit protective immunity to infections by a number of intracellular pathogens, including malaria pathogens (30, 32, 40). We observed in the present study that mice infected with *H. polygyrus* and immunized against malaria exhibited a significantly greater IL-10 response than mice either immunized with malaria vaccine or infected with the nematode alone exhibited. The increased IL-10 production in immunized nematode-infected mice may simply have been the result of an additive effect of IL-10 responses to both malaria immunization and *H. polygyrus* infection. It is also possible that preinfection with *H. polygyrus* potentiates the IL-10 response to malaria vaccine. Immunization with malaria vaccine did not induce a significant TGF-β1 response. However, high levels of bioactive TGF-β1 were observed in *H. polygyrus*-infected mice during the course of immunization. Furthermore, spleen cells from *H. polygyrus*-infected mice produced high levels of TGF-β1 in response to stimulation with *H. polygyrus* antigen. These findings suggest that *H. polygyrus* infection promotes a TGF-β1-producing Th3 regulatory T-cell response. Studies are currently in progress to investigate this possibility. The high level of TGF-β1 and the enhanced production of IL-10 in *H. polygyrus*-infected mice may inhibit the production of IFN-γ and protective antibodies required for vaccine-induced Th1-dependent protective immunity against malaria.

In summary, we demonstrated that concurrent nematode infection suppressed Th1-associated immune responses to antimalarial immunization and, consequently, reduced the protective efficacy of the vaccine. Although the Th2 cytokine responses induced by *H. polygyrus* may be involved in the modulation of vaccine-induced antimalarial immunity, the increased production of the regulatory cytokines TGF-β1 and IL-10 associated with nematode infection may represent an alternative mechanism for helminth-mediated immunosuppression. Importantly, our results show that deworming may be an effective strategy for improving the efficacy of malaria vaccines in humans living in areas where malaria and helminth infections coexist.

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